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Last Name	First Name	Middle Initial	Residence (City/State/Country)
FARIS	GREGORY	W.	MENLO PARK, CA UNITED STATES
KOTZ	KENNETH	T.	PALO ALTO, CA UNITED STATES
NOBLE	KYLE		SAN ANTONIO, TX UNITED STATES

Title: **"OPTICAL MICROFLUIDICS "**

Enclosed are:

☒ 10 pages of the specification (including description)
☒ 3 sheet of drawings
☐ __ page Abstract
☒ A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27.
☒ The invention was made by or under a contract with the following agency of the United States Government: SPACE AND NAVAL WARFARE SYSTEMS CENTER under Government contract number N66001-01-C-8057.

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Respectfully submitted,

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STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.27(a)(3))--NONPROFIT ORGANIZATION

Applicant: SRI INTERNATIONAL
Application No.: Not Yet Known
Filed: January 23, 2004
Title: OPTICAL MICROFLUIDICS

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SIGNATURE  DATE January 23, 2004

Attorney Docket No. US-5080-2P

U.S. PROVISIONAL PATENT APPLICATION

OPTICAL MICROFLUIDICS

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Optical Microfluidics

K. T. Kotz, K. A. Noble, & G. W. Faris

Much of the work on microfluidics to date has involved production of patterned fluidic circuits,[1,2] a relatively direct analogy with microelectronics. An alternative approach involves unconfined movement of individual droplets. While fluidic circuitry is the microscopic equivalent of tubing or pipes, droplet motion is a closer analogy to the individual droplet delivery obtained with pipettes, the mainstay of the macroscale chemistry and biology laboratory. Here we report a new method for control of small droplets based on the thermal Marangoni effect using laser heating. Using a single fluidic system, droplets covering 5 orders of magnitude in volume have been moved (~1.7 μL to 14 pL), with speeds of up to 3 mm/s. When two droplets are brought into contact, they spontaneously fuse and rapidly mix (< 33 ms). We have performed a simple chemical assay using horseradish peroxidase and a chromogenic substrate (ABTS), and we readily detect as little as ~125 attomoles of reacting enzyme using an absorption-based assay. This approach should be applicable to a variety of applications in chemistry and biology using small volumes and large numbers of samples.

Surface forces are the primary challenge for moving droplets on a solid surface. The strength of adhesion is indicated in part by the contact angle. Figure 1(a) shows a 1.8 mm diameter water droplet on a polystyrene surface immersed in decanol. The contact angle between the droplet and the surface (θ_c) is close to 180 degrees, indicating relatively little

surface adhesion. When a force is applied to the droplet, the symmetry between the contact angles is broken. The difference between the advancing and receding contact angles, or contact angle hysteresis, leads to a net force counteracting the applied force. The force required to move the droplet scales with the contact perimeter and the contact angle hysteresis.[3] Although we have successfully moved droplets using radiation pressure forces (optical traps or optical tweezers),[4] we find it is preferable to use surface tension forces to move the droplets.

Surface tension and surface energy generally drop as temperature is increased.[5] If a thermal gradient is applied along a droplet, the droplet moves toward the colder region to minimize the total surface energy, an effect called the thermal Marangoni effect. This effect can lead to formation of fingers extending from a pool of liquid onto a cooler surface. Control of these fingers has been achieved using regions of varying surface energy[6] or, recently, optical heating to produce patterned variation in surface energy.[7] Thermal gradients have also been used to produce rapid motion of droplets in combination with a surface energy gradient[8] and to flow droplets in a channel.[9] Similar surface tension imbalances generated with electric fields have also been used to move droplets.[10,11] Light forces have been used for fluidic control using photoisomerization to change the surface energy[12] or through optical traps either through direct manipulation of liposomes[13,14] or through optical manipulation of colloidal microspheres.[15] We show for the first time how a laser beam may be used to move droplets using the thermal Marangoni effect.

Aqueous droplets were deposited in an organic phase of 1-decanol on top of a standard polystyrene petri dish by two different methods. The first was by dragging the tip of a 34 gauge needle (100 μm inner diameter) across the bottom of the petri dish. The second was though the direct ejection of small droplets into the organic phase from a

standard inkjet print head (HP 51625a). We used the former method to produce droplets ranging in size from 40-1500 μm in diameter, while the latter method was capable of reproducibly generating aqueous drops on demand with diameters of 30-40 μm.

We move the droplets via an induced surface energy gradient, shown schematically in Fig. 1. We focus 10-200 mW of green light from an argon ion laser onto the imaging plane of an inverted microscope, controlling the position of the focus in the plane with a motorized mirror. The droplets contain an aqueous dye (FD&C Red No. 40 and Red No. 3, McCormick & Co. Inc.) that is diluted to a typical absorbance of 2 per centimetre at the laser wavelength. The absorption forms a temperature gradient across the drop (Fig. 1b), which in turn induces a surface energy gradient on the droplet surface via the Marangoni effect that is sufficient to move the droplets. Unlike prior thermal Marangoni-driven fluidics,[7-9] the surface tension forces here arise at a fluid/fluid boundary[16,17] rather than the fluid/solid boundary. It should be noted that the decanol influences the water/surface contact angle and also acts to control the evaporation of the droplets. Although we use a dye in the droplet for this work, it is not required. An alternative would be to add a dye to the surrounding fluid, or, preferably, use a laser that is selectively absorbed by either the droplet or the surrounding fluid so that no dye is required at all.

Using this simple method, we have been able to move droplets as large as 1.5 mm and as small as 30 μm in diameter. This corresponds to a range of volumes that spans 5 orders of magnitude (~1.7 μL to 14 pL). The speed of droplet movement was limited by the slew rate of the motorized mirror mount that steers the laser beam. By physically translating the stage, however, we moved the droplets at speeds of up to approximately 3 mm/s, which corresponds to 15 diameters/s for a 200 μm droplet. By scaling observations of gravity-driven motion of 3 μL droplets when the substrate is tilted, we

estimate the force required to overcome contact angle hysteresis for a 100 μm diameter droplet is approximately 10 nN. Calculating the Stokes (viscous) drag on a sphere including the Faxen wall correction,[17,18] we find a force of roughly 100 nN is required to move a 100 μm diameter droplet at 3 mm/s, so viscous drag dominates over forces from contact angle hysteresis in this case. Calculations of the temperature distribution in the droplet indicate typical temperature gradients of roughly 10^3 °C/cm, in agreement with the gradient required to produce the 100 nN force above[16,17] and yielding a temperature rise across the width of the droplet of roughly 10 °C.

Since the temperature gradient within the droplet is relatively large, the influence of this gradient on the fluid inside the droplet should be considered. There are two primary physical effects associated with thermal gradients: (1) convection and (2) mass transport effects (thermophoresis, thermal diffusion, or the Ludwig-Soret effect), which generally cause larger molecules or particles to move toward cooler regions. Internal convection velocities will be similar to the translation velocity,[16] or roughly 1 mm/s. In a 100 μm diameter droplet, this velocity produces a shear rate of about 10 s-1, which is far too small to damage molecules. By comparison, the shear rates used for breaking DNA molecules are about are about 10^4 times higher.[19] Thermal-gradient-driven mass transport can produce two effects: (1) a transient mass flow and (2) a concentration gradient in steady state.[20] Using, for example, the thermal diffusion coefficient for DNA (which is independent of DNA length),[21] and a thermal gradient of 10^3 °C/cm, we find a fraction solute flow rate of roughly 10 μm/s, too small to cause any adverse effects. The steady state concentration gradient scales with the Soret coefficient.[20] Taking as an example the Soret coefficient for 5.6 kbp DNA,[21] we find that the local concentration of the DNA will be reduced by a factor of about 4 in the hot region for a 10 °C temperature change. Such concentration gradients are unlikely to cause damage in most cases. If desired, the

temperature rise could be reduced by using a more uniform heating, for example, by sweeping the laser beam across the trailing edge of the droplet rather than using a single point focus, or using a surrounding liquid with lower viscosity to lower viscous drag. On the other hand, thermal gradients can also be beneficial. For example, the combination of thermal gradients and convection has led to a concentration enhancement of a factor of 60 for laser heating in a 50 μm channel,[21] which can enhance detection of small signals. Alternatively, the thermal cycling associated with convection can be used for rapid PCR,[22,23] which requires a temperature change of roughly 30 °C.

We observe very interesting behaviour from the small microdroplets following optical manipulation. The first is that two droplets spontaneously fuse upon contact with one another (Fig. 2). Droplet volume is conserved in each of the observed fusions, as expected. In addition, the droplets exhibit extremely rapid mixing following fusion, as shown in Fig. 2. As seen in the figure, the droplets have completely mixed in a time shorter than the time between video frames (33 ms). This spontaneous mixing appears to be a caused by the fusion process, and not by diffusion. For solutes with diffusion coefficients ranging from 5-0.5 x 10^{-6} cm^2/s,[24] it would take roughly 10 to 100 s to mix across 100 μm by diffusion alone. This rapid mixing is an important observation, because long mixing times often have been a challenge for microfluidics in channels.[2,25]

The mixing in this case is driven by the change in surface energy during droplet coalescence. The fused droplet has a lower surface area, and hence lower surface energy than the two droplets prior to fusion. The change in surface energy is largely converted to kinetic energy, causing droplet oscillation that is ultimately damped by viscosity. Using theory valid for small oscillations[26] and the water/decanol interfacial surface tension,[27] we

find that 100 μm diameter water droplets in decanol have an oscillation frequency of ~1.5 kHz and a damping time of ~110 μs.

One can define a characteristic velocity for this process by equating the change in surface energy from droplet fusion with the kinetic energy of the droplet volume. Indeed, observations of the dynamics of merging droplets[28] shows contact surface velocities similar to this characteristic velocity. For 100 μm diameter water drops in decanol, this characteristic velocity is ~50 cm/s, corresponding to a Reynolds number of ~70. The Reynolds number is a dimensionless quantity expressing the relative size of inertial and viscous forces; small Reynolds numbers are associated with difficulty in mixing. For flow over a cylinder, Reynolds numbers greater than about 2 are sufficient for formation of vortices.[29] Since the flow over a cylinder and the oscillations of coalescing droplets both involve direction-changing flow, it appears reasonable that vortices are formed for the droplets in our experiments. The convective motion of vortices would greatly enhance the mixing process. The internal Marangoni convection patterns[16] driven by the laser beam also will contribute to mixing, although the velocities are roughly a hundred times lower.

We have also performed initial experiments generating and moving aqueous droplets at the liquid-liquid interface of decanol and fomblin (a perfluorinated silicone oil). The three-component liquid system does not exhibit contact angle hysteresis, thus making it easier to move droplets. However, the lack of contact angle hysteresis allows the droplet movement to be adversely affected by convection currents or Brownian motion (for small droplets).

One realistic goal for any microfluidic application is the ability to perform a useful chemical assay. Fig. 3 shows the results obtained for a simple enzymatic assay carried out

using the optical microfluidic techniques described above. One droplet contains the enzyme, horseradish peroxidase, in phosphate buffer (0.1M pH 6.2), and the other contains an excess of the chromogenic substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-Sulfonic acid) diammonium salt (ABTS), and hydrogen peroxide. Upon mixing, the enzyme reacts with the substrates, and oxidizes the ABTS, resulting in a dark green droplet. Our observations are made with a long pass (red) filter, and thus the green droplet appears black in our images. In order to demonstrate the general usefulness of this technique we have moved both droplets of ABTS and HRP. The reaction is observed regardless of which drop is moved, demonstrating that we are not heating the droplets above the irreversible denaturing point of HRP. The transmission through the reacting drop exhibits smooth single-exponential decay as is expected for this reaction with a large excess of substrate. We have observed reaction in droplets as small as 40 μm in diameter, and at the concentrations used (~3.7 μM), this corresponds to ~125 *attomoles* of reacting enzyme. It should be stressed, however, that we have not fully optimised the above assay. The signal to noise (S/N) ratio, defined as the ratio of the change in transmission to the fluctuations in transmission versus time, for the lowest enzyme concentration (150 nM in 200 μm drops) studied was approximately 50. A reasonable lower limit to the S/N of ~3 would correspond to a reduction in signal by a factor of 20. This could be accomplished by reducing droplet diameter from 200 μm to 10 μm (the absorption signal scales linearly with the droplet diameter), corresponding to detection limits of *zeptomoles* of enzyme, a remarkably small quantity for an assay based on optical absorption. Much better detection limits should be possible with fluorescence-based assays.

In this letter, we have presented a new approach to microfluidics based on the optical control of thermal gradients. When compared with current lab-on-a-chip techniques, assays based on individual droplet movement eliminate the requirement of valves and pumps,

assays may be rapidly reconfigured on the fly, random access to substrate sites is easily accomplished, no on-chip "real estate" is devoted to optical and electrical circuitry, and the substrate, generally a disposable, does not require expensive and time-consuming fabrication. The simplicity of this technique should lend itself to a number of applications such as to manipulate and study very small fluid volumes (microfluidics and nanofluidics), to perform very large numbers of parallel assays (high throughput screening), to perform stochastic studies on small samples, or to perform molecular analysis on large numbers of isolated cells.

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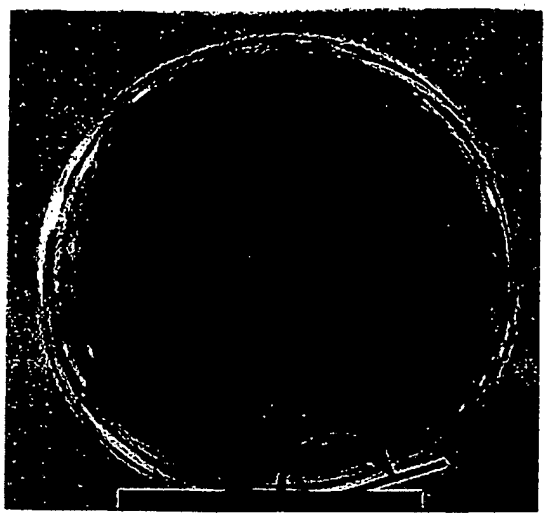
Figure 1(a) Photograph of the equilibrium contact angle (θ_e) for a drop of water in decanol on a polystyrene surface and **(b)** the schematic diagram of the experimental apparatus used to move the water droplets.

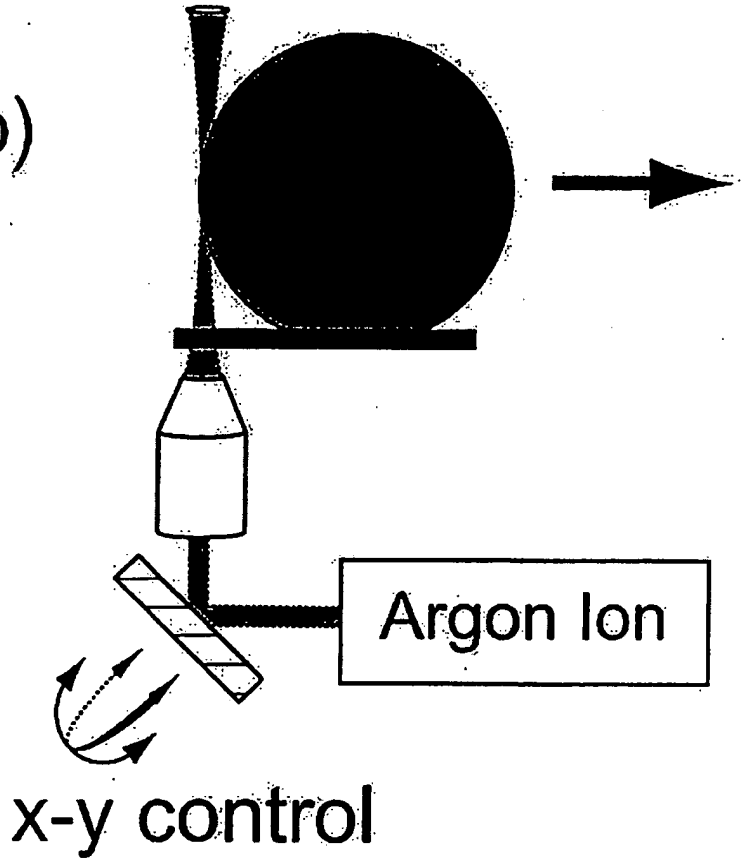
Figure 2 Sequence of two subsequent video frames (30 frames/sec) demonstrating the fusion and mixing of two different droplets. Images are taken through a long pass (red) filter to reduce the laser intensity. **a**, A dye filled droplet (lower left) has been moved by the focus of the laser (black arrow) next to a droplet containing black India ink. The scale bar is 100 μm. **b**, The two combined droplets in the next video frame, 0.03 s later. Droplet fusion and mixing takes place on a timescale of less than 0.03 seconds. See supplemental information for a video of this fusion and mixing.

Figure 3 Images extracted from a video of a chemical assay viewed through a long-pass (red) filter. The scale bar in a represents 100 μm. **a**, Two sets of droplets, one containing ABTS and hydrogen peroxide in phosphate buffer (large drops) and the other containing HRP and dye in phosphate buffer (small drops). **b**, One of the small drops (grey arrow) has been moved by the focus of the laser beam (black arrow) adjacent to a drop containing ABTS. **c**, The video frame immediately following **b**. The two droplets have fused, but not yet reacted. **d**, Two minutes after the fusion of the droplets, clearly showing the change in absorption due to the oxidation of ABTS. A shortened video of this reaction is available in the supplemental information.

a)

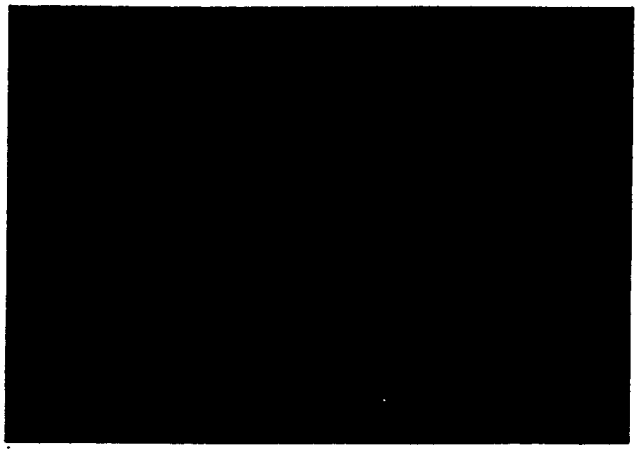


b)

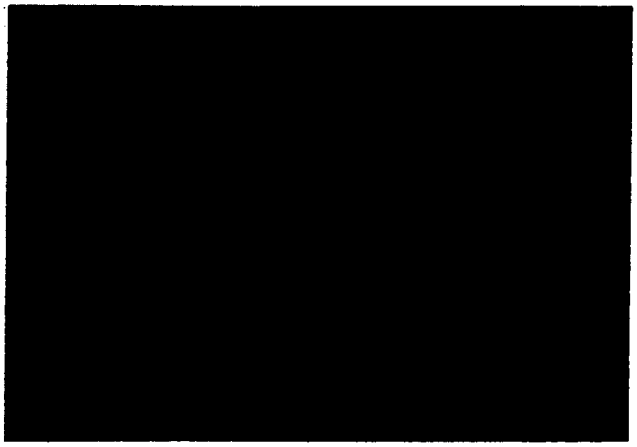


Kotz, Noble & Faris Figure 1

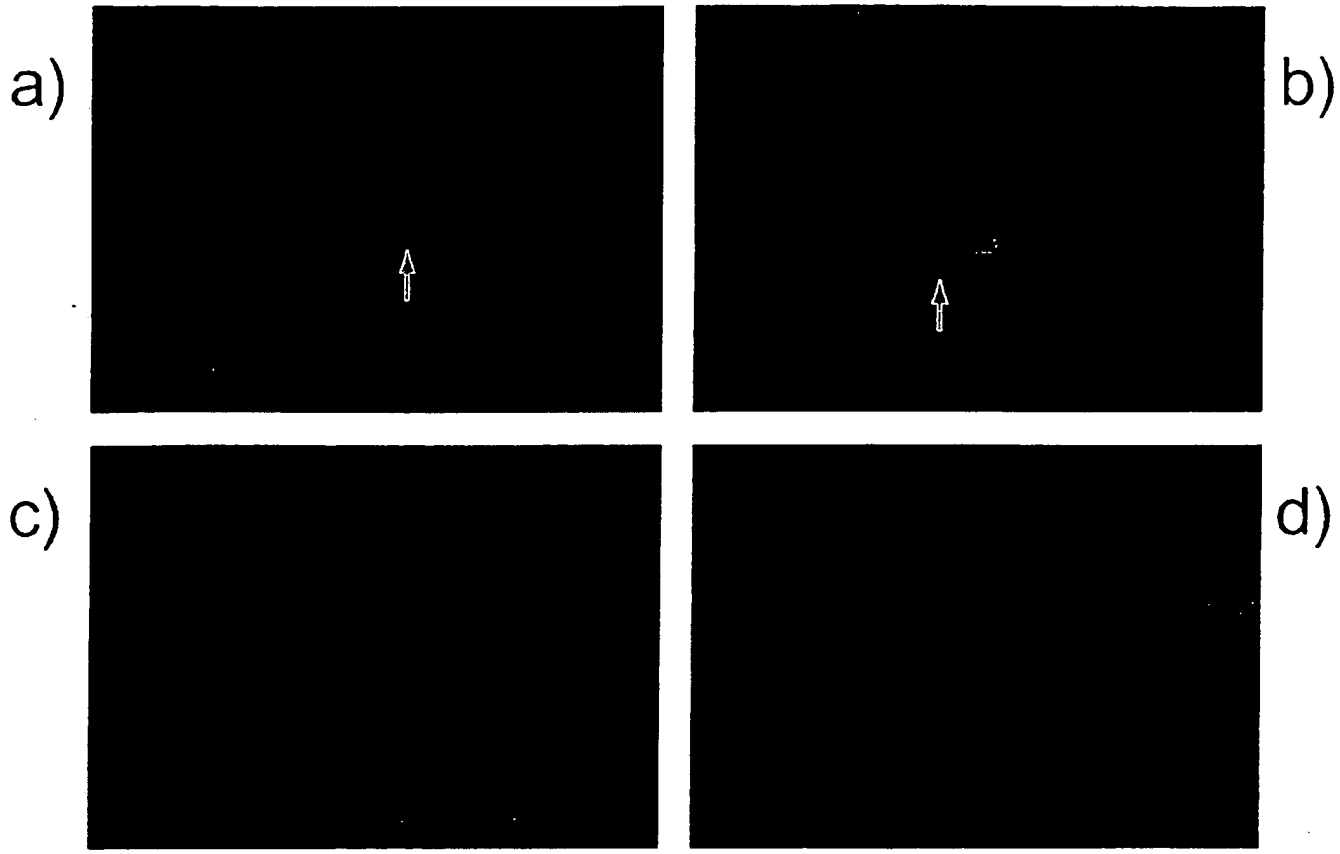
a)



b)



Kotz, Noble & Faris Figure 2



Kotz, Noble & Faris Figure 3

PATENT COOPERATION TREATY

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(PCT Administrative Instructions, Section 411)

To:

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Date of mailing *(day/month/year)* 12 April 2005 (12.04.2005)	
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Applicant SRI INTERNATIONAL et al	

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